

Nutrient agar shows *Pseudomonas aeruginosa* pigment.

- *Pseudomonas aeruginosa* produce yellow-green water-soluble exopigments (pyocyanin) that diffuse to the medium changing its color.



A Blood agar shows β hemolysis

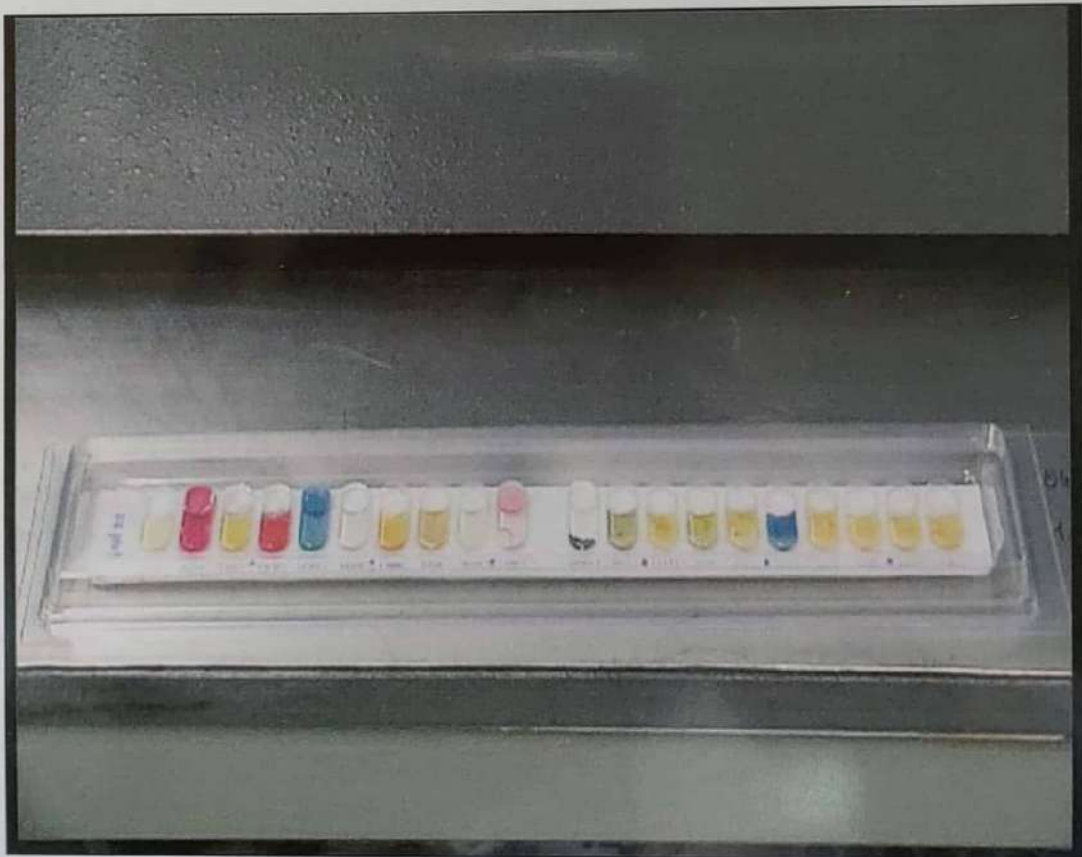
- It is a complete hemolysis of red blood cells.
- The colonies are surrounded by clear zone of hemolysis e.g. *Streptococcus pyogenes* and *Staphylococcus aureus*.



API System

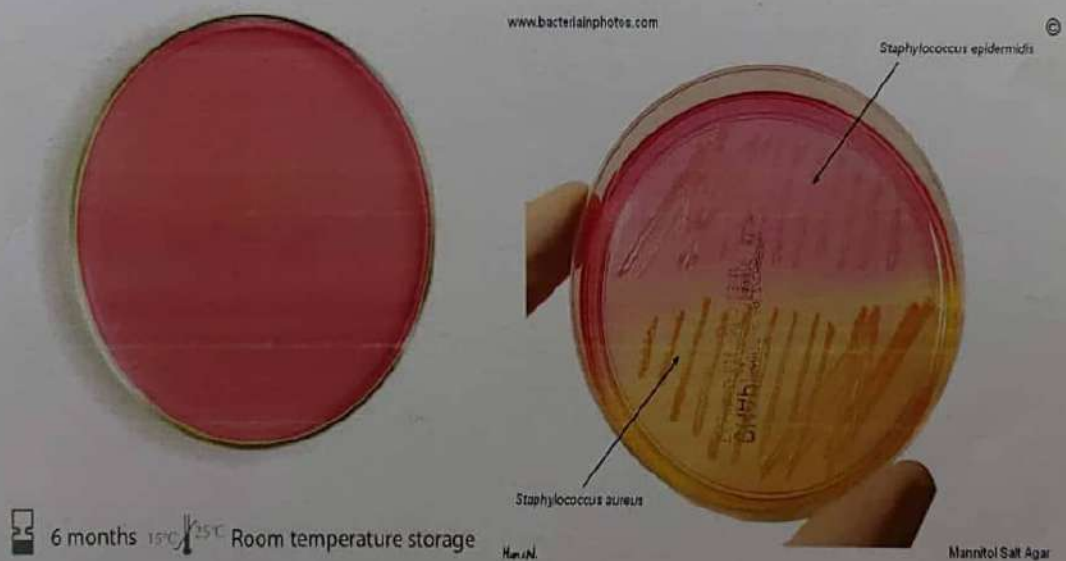
(Commercial system for identification of bacteria)

It is biochemical identification systems that readily available from commercial source e.g. API system. It is composed of plastic strips with cups containing dried reagents into which a suspension of the test organism is placed. A biochemical profile of the organism is obtained after 24 – 48 hs incubation at 37 °C in the form of colour changes. The profile is translated into a numerical code, which can read from a key (Profile Index).



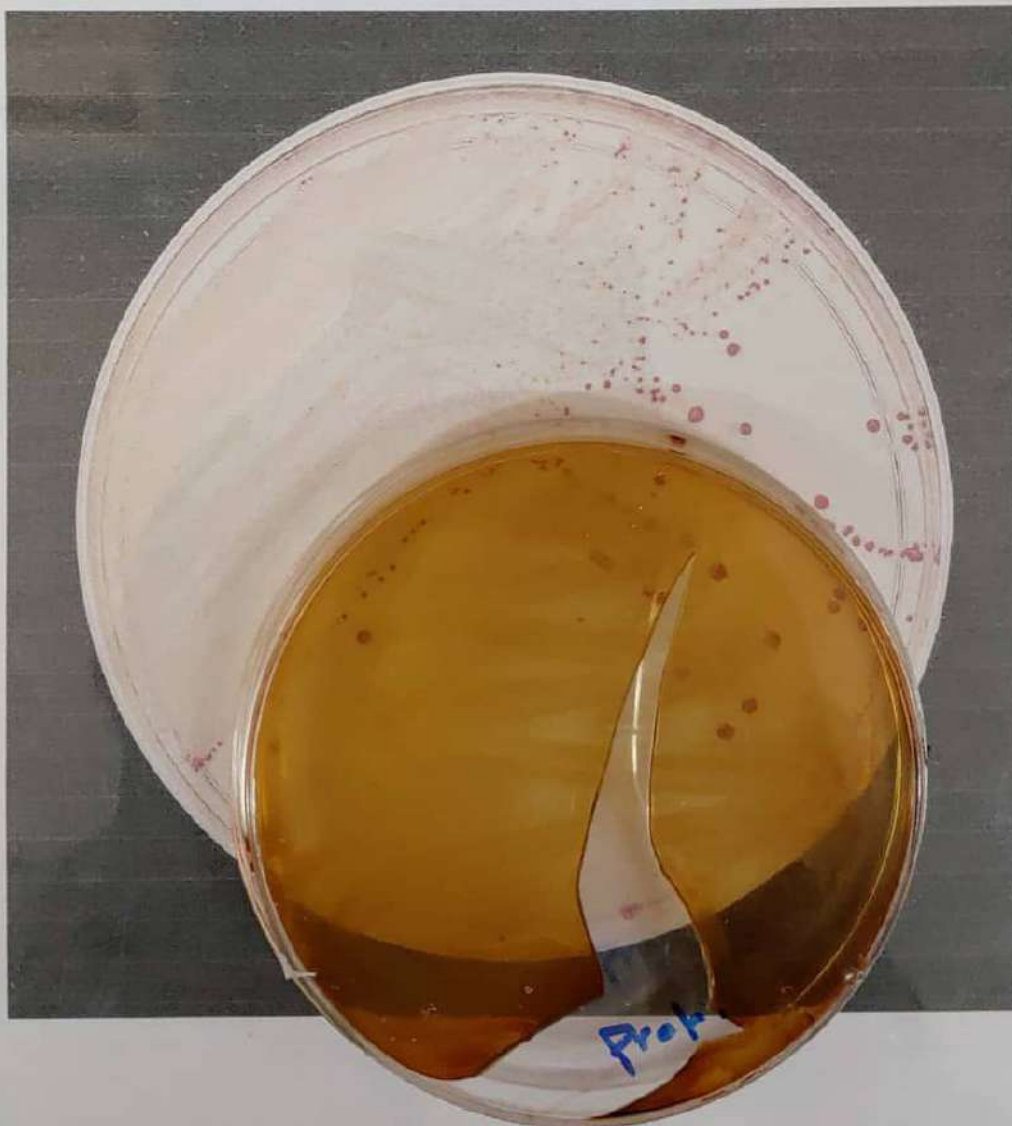
MANITOL SALT AGAR

- Selective agent : 7.5% NaCl
- Differential agent : mannitol to differentiate between manitol fermenters and nonfermenters
- PH indicator : phenol red



Non-lactose fermentation on MacConkey agar

- Non-lactose fermentation by producing pale yellow colonies.
- The non-lactose fermenters e.g. *Salmonella*, *Shigella* and *Proteus*.



Chocolate blood agar (Heated blood agar)

Composition: 5-10% sterile defibrinated sheep, or human blood is added to melted sterile nutrient agar at 55°C. Then the temp. is raised to 100°C for 2 min.

Sterilization: Nutrient agar is sterilized in the autoclave at 121°C for 20-30min, and the blood is taken under complete aseptic condition.

Appearance: It is an opaque brown solid medium. It is in the form of plates or slopes.

Uses: It is used for isolation of fastidious organisms e.g. *Haemophilus influenzae*, pathogenic *Neisseria* and *Pneumococci*.

- Heating the red blood cells, leads to their rupture and liberation of nutrients e.g. X factor and V factor which essential for growth of these organisms.



Uninoculated chocolate agar plate

Thiosulphate citrate bile sucrose (TCBS) agar

Composition: It is alkaline agar medium contains thiosulphate, citrate and bile as selective substances. It contains also sucrose as test sugar and bromothymol blue as indicator that give yellow color in acidic pH.

Appearance: It is a green semi-transparent medium.

Uses: It is a selective and indicator medium for the isolation of *Vibrio cholerae*. *Vibrio cholerae* produces yellow colonies due to fermentation of sucrose with acid production.



Lactose fermentation on MacConkey agar

- lactose fermentation by producing pink colonies.
- The lactose fermenters e.g. *E. coli*, *Klebsiella* and *Citrobacter*.



Nutrient agar

Composition: 2-3% agar dissolved in nutrient broth. Agar is obtained from special seaweeds in the form of powder or shreds. Dissolution is aided by heating at 100°C and the pH is adjusted to 7.5.

Sterilization: In the autoclave at 121°C for 20-30min.

Appearance: It is a yellowish semi-transparent solid medium (at temp. below 40°C). It is in the form of plates, slopes, and deep agar.

Uses of nutrient agar:

1. It is a base of other media e.g. blood agar and chocolates agar media.
2. Nutrient agar in plates is used for isolation, purification and identification of microorganisms.
3. The slopes are used for preservation of pure cultures.
4. The deep agar is used for cultivation of anaerobic organisms.



Sugar Media

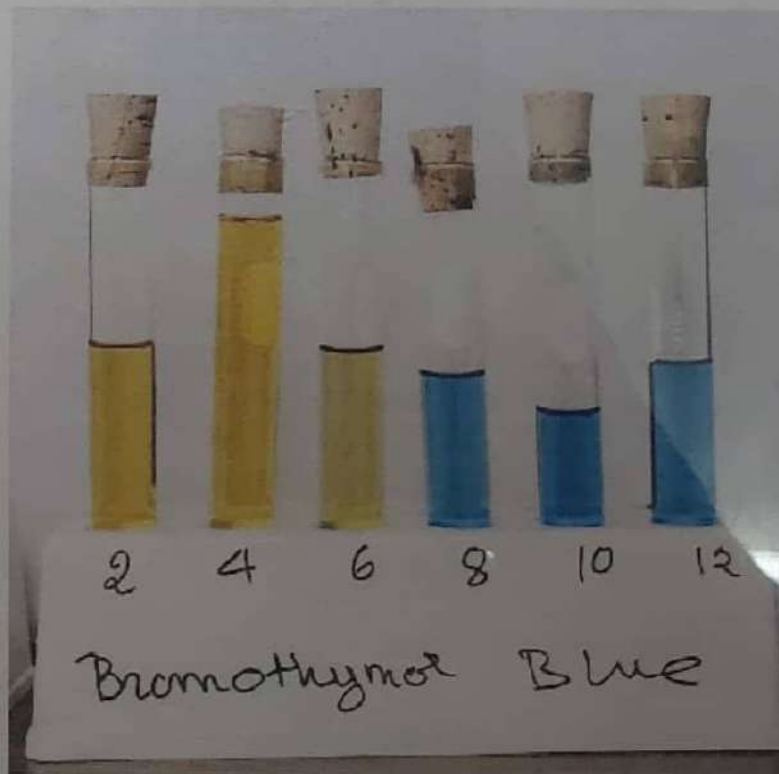
Composition: Peptone water, 1% sugar (glucose, lactose, maltose, mannite and sucrose), pH indicator (bromothymolblue), inverted Durham's tube.

Sterilization: Koch's sterilizer at 100 C for 20 min. on three successive days (Tendallization).

Uses:

To test the biochemical activity of bacteria on carbohydrate.

- Fermentation with production of acid only: yellow color e.g. salmonella typhi.
- Fermentation with production of acid + gas: yellow color + gas bubbles in Durham's tube e.g. E. coli.



Peptone water

Composition: 1% peptone and 0.5% NaCl dissolved in water, pH is adjusted to 7.5.

Sterilization: in the autoclave at 121°C for 20-30min.

Appearance: It is clear colorless fluid.

Uses of peptone water:

1. As a base for other media e.g. sugar media.
2. Test for indole production.
3. Alkaline peptone used as an enrichment medium for isolation of *Vibrio cholera*.



MacConkey agar

Composition: Peptone, agar, bile salt, lactose(test sugar) , neutral red indicator.

Sterilization: in the autoclave at 121°C for 20-30min.

Appearance: It is a reddish transparent solid medium.

Use:

- It is a selective medium for enterobacteriaceae.
- Differentiate between lactose fermenters (pink colony), non lactose fermenters (yellow colony).



Swarming growth of proteus

- Motile Proteus strains produce a characteristic growth over the surface of nutrient.
- Swarming appears as a thin, colorless, transparent film extending from the margins of a young colony and spreading in several waves until most or all the surface of the culture plate is covered.
- Swarming is inhibited by:
 - Bile salts e.g. MacConkey agar and DCA.
 - Increasing the concentration of agar.
 - Addition of chloral hydrate to the medium.



Nutrient broth (Meat infusion broth)

Composition:

- 500 gm fresh, minced, red meat free from fat is added to 1-liter tap water.
- The meat is left to infuse in the refrigerator over night, then cooked in the steamer for 2 hrs and filtered.
- 1% peptone and 0.5% NaCl are added to the filtrate. The pH is adjusted to 7.5.

Sterilization: In the autoclave at 121°C for 20-30min.

Appearance: It is clear yellowish fluid.

Uses of ^{Nutrient broth:} peptone water:

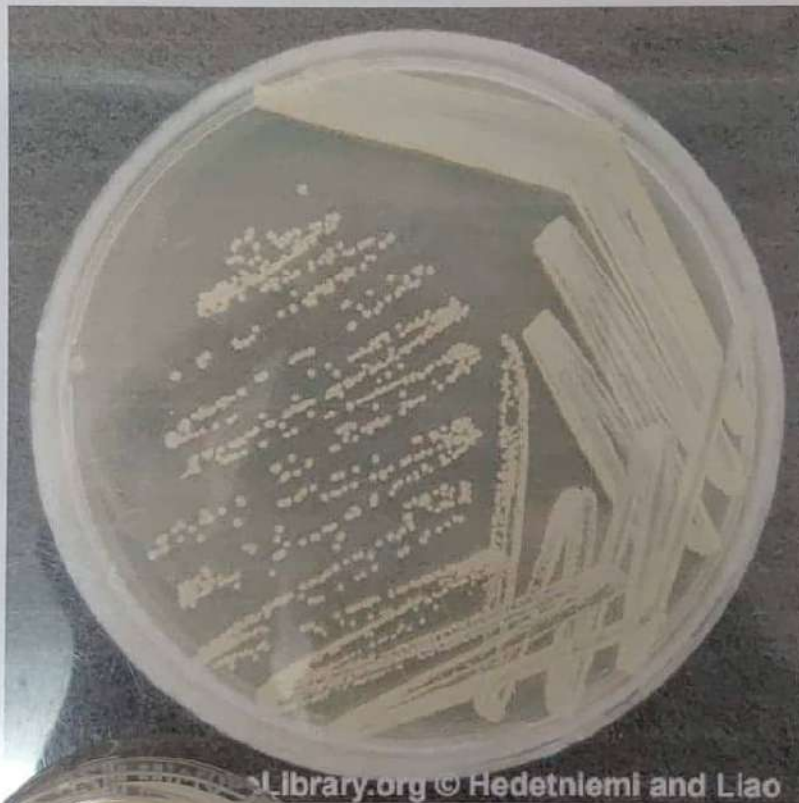
1. It is the base of most of the culture media e.g. nutrient agar and gelatin medium.
2. It is used for blood culture.
3. To test for coagulase production by Staph. Aureus.



Nutrient agar shows endopigment

Colored colonies: due to production of water insoluble , non – diffusible endopigments.

e.g. *Staphylococcus aureus*: produce golden yellow pigments.



Gas Pak Anaerobic Jar

It is a clear plastic jar with an airtight lid. The catalyst composed of palladium pellets held in a wire mesh fitted underneath the lid. Anaerobic condition is achieved by the use of commercially available H₂ and CO₂ generator envelope. 10 ml of water is added to the envelope. The envelope is placed with the inoculated plates in the jar, which is tightly closed. The catalyst allows the combination of O₂ with H₂ to form H₂O, thus establishing complete anaerobiosis. Anaerobic indicator e.g. methylene blue is put in the jar to check that the catalyst and the GasPak envelope are functioning properly.



A blood agar shows α hemolysis

- It is a partial hemolysis of red blood cells.
- The colonies are surrounded by a green discoloration e.g. *Viridans streptococci* and *streptococcus pneumonia*.



Loeffler's serum medium

Composition: Three parts of horse or ox serum + 1 part of glucose broth.

Sterilization: Serum is sterilized by filtration and glucose broth by Koch's steamer then the medium is solidified in hot air inspissator at 75°C for 2 hours on two successive days.

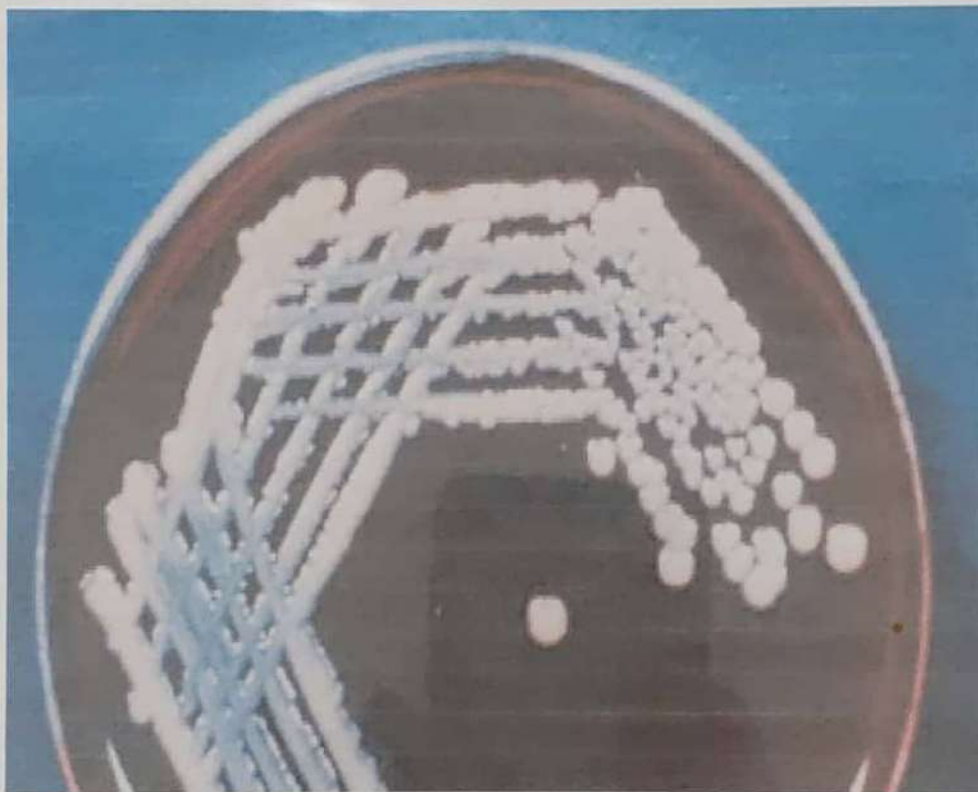
Appearance: It is an opaque whitish medium, distributed in tubes of screw-capped bottles in the sloped position.

Uses:
For cultivation of *Corynebacterium diphtheria*.



A blood agar shows γ hemolysis

- There is no effect on the blood.
e.g. *Staphylococcus albus* and *Neisseria*.



Lowenstein-Jensen medium

Composition: It is a modified egg medium containing malachite green, which has inhibitory effect on the growth of organisms other than mycobacteria.

Sterilization: The medium is solidified in a hot air inspissator at 80°C for one hour.

Appearance: It is opaque green in colour, distributed in tubes of screw-capped bottles in the sloped position.

Use: It is selective and enriched medium; used for isolation of Mycobacterium tuberculosis from specimens contaminated with commensal bacteria e.g. sputum, urine and stool.



Blood agar

Composition: 5-10% sterile defibrinated sheep, or human blood is added to melted sterile nutrient agar at 55°C.

Sterilization: Nutrient agar is sterilized in the autoclave at 121°C for 20-30min, and the blood is taken under complete aseptic condition.

Appearance: It is an opaque red solid medium. It is in the form of plates or slopes.

Uses of blood agar:

1. It is an enriched medium that supports the growth of most delicate organisms e.g. streptococcus pyogenes.
2. It is an indicator medium showing haemolytic properties of bacteria. It differentiate the organism according their action on the blood:
 - Complete Hemolysis (β -hemolysis): e.g. Staphylococcus aureus & Streptococcus pyogenes.
 - Incomplete Hemolysis (α -hemolysis): e.g. Viridans streptococci and streptococcus pneumoniae.
 - No effect on blood (γ -hemolysis): as staphylococcus albus.

